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Artículo de Revisión

Oxytetracycline and gentamicin: two clinically-relevant antimicrobials widely used by Costa Rican farmers. Implications of their use outside clinical settings and request for action.

César Rodríguez Sánchez

Sección de Bacteriología General and Centro de Investigación en Enfermedades Tropicales. Facultad de Microbiología. Universidad de Costa Rica.

Resumen

Esta mini-revisión se relaciona con el uso de la oxitetraciclina y la gentamicina, dos antimicrobianos de aplicación en medicina humana, en la horticultura y otras actividades productivas. El manuscrito inicia con una breve descripción del mecanismo de acción y uso clínico de ambas drogas. Seguidamente, el autor aborda los principales mecanismos de resistencia adquirida que han sido descritos hasta la fecha y algunas implicaciones de su mal uso y abuso en escenarios no hospitalarios. En este sentido, se presentan reportes de la ocurrencia de bacterias resistentes y genes de resistencia que demuestran la existencia de un riesgo y que justifican la instauración de programas de monitoreo y de impacto ambiental.

Palabras clave: Oxitetraciclina, gentamicina, resistencia antimicrobiana, agricultura.

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Abstract

This mini-review deals with oxytetracycline and gentamicin: two clinically-relevant antimicrobials that find extensive usage in horticulture and other productive activities. The manuscript begins with a few words on their mechanism of action and clinical utility. Thereafter I address the most important mechanisms of acquired or intrinsic resistance described to date and some implications of the unrestricted use of these drugs outside clinical settings. A number of reports on the occurrence of antimicrobial-resistance genes and antimicrobial-resistant bacteria in agroecosystems illustrates that a threat exists and justifies the plea for monitoring programs and environmental risk assessments with which the article concludes.

Keywords: oxytetracycline, gentamicin, antimicrobial-resistance, plant agriculture.

Mechanism of action and clinical utility of oxytetracycline and gentamicin

Sobin *et al*. [1] were the first to purify oxytetracycline from fermentation broths inoculated with *Streptomyces rimosus*. This drug belongs to the tetracyclines, a group of broad-spectrum antimicrobials that inhibit protein synthesis by blocking the binding of aminoacyl-t-RNAs to the A-site of the ribosome [2]. In Gram-negative bacteria, tetracyclines move passively through membranes via porin channels and accumulate in the periplasmic space. Later on, their movement across the cytoplasmic membrane requires energy provided by the ΔpH of the proton motive force [3].

The tetracyclines exhibit bacteriostatic activity against a wide variety of bacteria and certain protozoans (*Entamoeba histolytica, Giardia lamblia, Leishmania major, Trichomonas vaginalis,* and *Toxoplasma gondii*). They are particularly helpful in the treatment of atypical pneumonia syndromes, chlamydial genital diseases, Lyme disease, ehrlichiosis, and infections by *Mycoplasma* sp. [4]. Moreover, oxytetracycline is useful for the topical treatment of ocular disorders [5], rosacea, and facial acne [6]. This group of antibiotics is contraindicated during pregnancy and in the childhood due to adverse side effects such as dental staining and interference with bone growth. In addition, the tetracyclines may instigate photosensitivity as well as drug and food interactions that disturb normal absorption processes in the gastrointestinal tract.

Gentamicin was first isolated from a strain of *Micromonospora purpurea* in 1963 [7]. At concentrations below 2 μg ml^{-1}, it inhibits the initiation step of protein synthesis through attachment to a conserved rRNA sequence located

near the spot of codon-anticodon recognition in the A-site of the ribosome [8]. At higher concentrations, it induces misreading and accumulation of abnormally long polypeptides due to destabilizing effects on tRNA-mRNA interactions [9]. Passage of gentamicin across the outer membrane of Gram-negative bacteria involves passive disruption of Mg^{+2} bridges between adjacent lipopolysaccharide molecules. Thereafter, its transport across cytoplasmic membranes depends on electron transport. Penetration through porin channels is unlikely because of their large size [10].

The aminoglycosides display concentration-dependent, bactericidal action against a wide range of aerobic Gram-negative bacilli, staphylococci, and certain Actinobacteria [11]. Consequently, their commercialization rapidly revolutionized the treatment of *Mycobacterium tuberculosis* [12] and *Pseudomonas aeruginosa* [13]. These antimicrobials exhibit some advantageous features, such as poor gastrointestinal absorption, reduced penetration into the cerebrospinal fluid, negligible binding to plasma proteins, and fast excretion by uncompromised kidneys [14]. However, they should be prescribed with caution because of their recognized oto- and nephrotoxicity [15]. From this large family of antimicrobial agents, the low cost and reliable activity of gentamicin give good reasons for its prescription [16].

Mechanism of resistance to oxytetracycline and gentamicin in the bacterial world

Until now, four different mechanisms confer acquired resistance against the tetracyclines [17]. Namely, reduction of their intracellular concentration by means of energy-dependent efflux proteins of the major facilitator superfamily (efflux proteins; EP), protection of the ribosome by soluble proteins from the superfamily of GTPases (ribosomal protection proteins; RPP), enzymatic modification of the antibiotics in the presence of oxygen and NADPH (tetracycline modifying enzymes; TME), and a further, poorly understood, mechanism. The 23 EP known so far, which catalyze the exchange of a proton for a tetracycline-cation complex against a concentration gradient, consist of 12 (Gram-negative bacteria) or 14 (Gram-positive bacteria) membrane-spanning helixes separated by short regions of hydrophilic amino acids [18]. Intriguingly, 16 genes from this family of resistance determinants (~70%) are restricted to Gram-negative bacteria [17]. The second most prevalent mechanism of acquired resistance to tetracyclines is due to RPP. Considering their structural resemblance to the elongation factors EF-Tu and EF-G, RPP seem to cause an allosteric disruption that releases tetracyclines from their binding sites and returns the ribosome to its standard post-translocational conformation [19]. Most genes for RPP have G+C% contents compatible with a Gram-positive origin. However, they are found in both Gram-positive and Gram-negative bacteria [17].

Besides the genetic elements discussed above, tetracycline resistance can be caused by differences in cell permeability [20] and porin expression [21], or as a result of the (over)expression of multi-drug resistance pumps [22]. To conclude, tetracycline-resistant pathogens of clinical importance rarely owe their limited susceptibility to mutations in their 16S rRNA genes [3].

As to gentamicin, resistance to aminoglycosides is mainly due to covalent modification of the antibiotics by a diverse collection of enzymes [23]. In this context, *O*-phosphotransferases (APH) and *O*-adenyltransferases (ANT) employ ATP to alter hydroxyl functions. *N*-acetyltransferases (AAC), on the other hand, make use of acetyl-coenzyme A to modify amino groups [10]. Structural modifications of gentamicin on positions 2" and 3' by APH, 2" by ANT, and 2', 3 and 6' by AAC result in a severe reduction of the ability of this drug to bind to its target RNA [24].

To conclude, further mechanisms of resistance to this drug arise from decreased uptake and/or accumulation of the drug. This has to do with differences in the organization of respiratory chains [25], alterations in cell membranes [26], or overexpression of drug efflux systems [27].

Application of oxytetracycline and gentamicin in plant agriculture and implications of their use outside clinical settings.

Although modest in relation to their application in human and veterinary medicine, streptomycin, oxytetracycline, and gentamicin are the most widely used antimicrobials in horticulture [28]. These substances are generally sprayed to the aerial parts of crops or to farmlands at concentrations ranging from 50 to 300 parts per million [29].

Calcium complexes or hydrochloride salts of oxytetracycline are internationally sold under the trademarks Glomycin®, Terrafungine®, Riomitsin®, Hydroxytetracycline®, Berkmycin®, Biostat®, Impercin®, Oxyatets®, Mycoshield®, and Agricultural terramycin® for control of fire blight of pears, pear decline, bacterial spot on peaches and nectarines, lethal yellowing of coconut palm, and lethal decline of pritchardia palm (U.S Environmental Protection Agency. Pesticide Fact Sheet 12/88). On the other hand, gentamicin sulfate is for the most part marketed for protection of tomatoes, potatoes, peppers and pome fruits [29]. This drug is formulated alone (Agry-Gent®, Química Agronómica de México, Chihuahua, México) or in combination with oxytetracycline (i.e. Bactrol® or Agry-Gent Plus®).

In our country, farmers employ agricultural supplements containing oxytetracycline and/or gentamicin without knowledge of the appropriate dosage. Moreover, the lack of clear national policies and monitoring

programs allows them to overlook the custody of qualitative and quantitative records of the applications done. These practices are worrisome because oxytetracycline retains partial activity when bound to soil particles [30, 31]. In addition, these drugs can accumulate in agricultural soils [32] or in crops subsequent to fertilization with manure [33]. Equivalent information is not available for gentamicin at present.

The genetic principles of adaptive evolution in prokaryotes, which largely differ from those of sexually reproducing eukaryotes by reason of the clonality and outstanding capacity of the former to transfer genetic material laterally [34], turn the development of resistances to these drugs into an inevitable outcome of their use. Thus, if clinically-relevant antimicrobials are heavily employed in polymicrobial compartments -such as the soil or the surface of plants-, crops treated with antimicrobials may expose consumers to antimicrobial-resistant bacteria selected in the environment. The occurrence of bacterial strains with similar protective mechanisms in farms and hospitals substantiate this assumption [35, 36].

Prevalence of antimicrobial-resistant bacteria and resistance genes in agroecosystems

Numerous investigations have demonstrated the occurrence of tetracycline- and gentamicin-resistance genes in bacteria from crops and farms from other latitudes [37, 38, 39, 40, 41, 42, 43, 44]. In Costa Rica, researchers from the Faculty of Microbiology have detected complex communities of culturable oxytetracycline- and gentamicin-resistant bacteria as well as several resistance genes and factors for their potential horizontal transfer on lettuce heads for human consumption collected in Zarcero and Cartago [45]. In addition, elevated amounts of tetracycline- and gentamicin-resistant bacteria have been found among gut bacteria of swine and mantled howler monkeys, and in bacteria from the phyllosphere of lettuce [46], coriander, cabbages, and sweet peppers.

The inexistence of surveillance programs, combined with the lack of application records, frustrates any attempts to estimate the real amounts of antimicrobials used by Costa Rican farmers. However, rough calculations suggest that the magnitude of tetracycline and gentamicin consumption in agriculture might exceed the quantities employed in human medicine by a factor of 200 or 700, respectively (García F., personal communication). With this contribution, I would like to propose that the release of antibiotics in agriculture should be restricted to cases where their need and utility has been systematically demonstrated. I also advise to start monitoring programs of antimicrobial resistance outside hospitals, to perform environmental risk assessments, and to keep records of antimicrobial acquisition or application by horticultural farmers. Otherwise, we could be jeopardizing the utility of drugs which have deeply improved the quality of life of many people around the globe.

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Correspondencia:

César Rodríguez Sánchez

Correo electrónico: cesar.rodriguezsanchez@ucr.ac.cr.
Teléfono: +506-2207-43-64.

